nucleotide, to the primer depends upon the identity of the unpaired nucleotide base in the nucleic acid template, and wherein when the target nucleotide is changed to any other type of nucleotide, a plurality of non-terminator nucleotides labeled with said detectable marker are sequence-dependently incorporated into the primer extension; and

(g) determining the presence of the other type of nucleotide at the predetermined position in the nucleic acid of interest by detecting the presence of detectable signal of the non-terminator nucleotides extended from the primer, without employing gel electrophoresis size separation method.

REMARKS

Claims 2-9 and 11-37 are pending in the application. Bases for the amendments to claim 37 can be found at, *inter alia*, page 4, lines 16-25 in the present specification, in which the primer strand is described as being extended by incorporation of a plurality of labeled nucleotides, and mutations can be detected without employing size separation methods. No new matter has been inserted into the application. Further, it is believed that no new issue is raised requiring further search or consideration as the amendments to claim 37 are directed to merely clarifying the presently claimed invention.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 2-38 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has objected to the recitation of "wherein the target nucleotide base in original form is not immediately adjacent on its 3' side to an identical base" in claim 37. This language does not appear in amended claim 37. Accordingly, this rejection has been overcome. Applicant notes that the removal of this language from claim 37 is not made to overcome a prior art reference, but rather is made so that the claims are merely further clarified.

The Examiner has also rejected claims 2-38 based on lack of antecedent basis for "the mutated base". Claim 37 has been amended to replace this phrase with "other type of nucleotide". Thus, this rejection has been overcome.

Rejection Under 35 U.S.C. § 102(b) Over Applied Genetics '545 (WO 96/30545)

Claims 2-15 and 23-38 have been rejected under 35 U.S.C. § 102(e) as being anticipated by AG '545. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Present Invention and Its Advantages

The present invention is directed to a method for detecting or quantifying a target nucleic acid in a sample by detecting signal from a plurality of labeled nucleotides incorporated into a primer comprising:

(a) selecting a nucleic acid having a target nucleotide base at a predetermined position in a template of a nucleic acid of interest;

(b) preparing an unlabeled primer complementary to a sequence immediately upstream of the target nucleotide base;

(c) treating a sample containing the nucleic acid of interest, if the nucleic acid is double-stranded, so as to obtain unpaired nucleotide bases spanning the specific position, or directly employing step (d) if the nucleic acid of interest is single-stranded;

(d) annealing the primer from (b) with the target nucleic acid from (c) under high stringency conditions to obtain a primer-nucleic acid duplex, wherein the target nucleotide base in the nucleic acid of interest is the first unpaired base immediately downstream of the 3' end of the primer;

(e) mixing the primer-nucleic acid duplex from (d) with a primer extension reaction reagent comprising: (i) three types of non-terminator nucleotides that are not complementarily matched to the target nucleotide, wherein at least one type of the non-terminator nucleotide is labeled with a detectable marker; and optionally (ii) one type of terminator nucleotide that is complementarily matched to the target nucleotide, wherein the terminator nucleotide is not labeled;

(f) performing the primer extension reaction by enzymatic or chemical means, wherein the incorporation of said non-terminator nucleotide and optionally, the terminator nucleotide, to the primer depends upon the identity of the unpaired nucleotide base in the nucleic acid template, and wherein when the target nucleotide is changed to any other type of nucleotide, a plurality of non-terminator nucleotides labeled with said detectable marker are sequence-dependently incorporated into the primer extension; and

(g) determining the presence of the other type of nucleotide at the predetermined position in the nucleic acid of interest by detecting the presence of detectable signal of the non-terminator nucleotides extended from the primer, without employing gel electrophoresis size separation method.

Advantages of the invention include: 1) capability of detecting all types of mutations in only one reaction tube without necessarily employing gel electrophoretic size separation methods; and 2) high degree of detection sensitivity by way of strong signal emitted due to incorporation of multiple labeled-nucleotides into the primer extension strand. These advantageous features provide an opportunity to use the invention to routinely, rapidly and accurately test for the presence of a genetic mutation in any clinic setting based on this simple inventive procedure. The invention is also easily adaptable to automation for screening a large number of samples.

Applied Genetics '545

AG '545 discloses analyzing a genetic mutation by hybridizing a primer to a target nucleic acid, extending the primer in the presence of a mixture of at least one deoxynucleoside triphosphate and at least one chain terminating dideoxynucleoside triphosphate, separating the primer, the mutant DNA-derived extension product and the wild-type extension product on the basis of their size, and identifying the mutant DNA-derived extension product and the wild-type extension product. Furthermore, the Examples disclose using a fluorescein-labeled primer (pages 31, 32 and 44). AG '545 also discloses that the primer is hybridized "somewhere proximal and 3' to the point of deviation" (page 18, lines 13-19). Moreover, in Figure 6 of AG '545, three terminator dideoxynucleotides and one deoxynucleotide are included in a reaction mixture, wherein the deoxynucleotide is complementary to the wild-type target nucleotide. AG '545 also requires a size separation method to sequence the mutant or wild type gene.

Applicant submits that AG '545 contains embodiments that are contradictory to the principles underlying the instantly claimed subject matter, and as a consequence, fails to be

applicable to the claimed subject matter. For instance, to illustrate the non-relevance of the AG '545 reference, in all of the Examples of AG '545, the oligonucleotide primer itself is pre-labeled with a fluorescent dye, which is inconsistent with the practice of the claimed invention. In contrast to AG '545, the presently claimed invention is directed to preparing an unlabeled primer complementary to a sequence immediately upstream of the target nucleotide base.

There are other differences. Whereas AG '545 allows for the primer to be hybridized somewhere proximal and 3' to the point of deviation, the presently claimed invention is directed to annealing the primer to the target nucleic acid such that the target nucleotide base in the nucleic acid of interest is the first unpaired base immediately downstream of the 3' end of the primer. The presently claimed invention relies on the primer being immediately adjacent to the target nucleotide. AG '545 fails to appreciate this point.

Moreover, AG '545 discloses using multiple terminator dideoxynucleotides. In contrast, the presently claimed invention is directed to mixing the primer-nucleic acid duplex with a primer extension reaction reagent comprising three non-terminator nucleotides and only one type of terminator nucleotide that is complementarily matched to the target nucleotide, in which the terminator nucleotide is not labeled. All of the above inconsistencies and incongruence with the instantly claimed method suggest that at a fundamental level, AG '545 teaches away from the invention or is totally irrelevant to the claimed invention. However, there is more.

In addition to the above, Applicant further notes that AG '545 fails to disclose or suggest which of the dideoxy- or deoxy- nucleotides should be labeled. Nowhere is it taught in AG '545 that the dideoxynucleotide should not be labeled and that the deoxynucleotide should be labeled as in the instant claims, or even that there should be even any sort of differentiation in the reporter moieties between that used for dideoxynucleotides versus deoxynucleotides. The

Examiner is reminded that in the instantly claimed invention, the dideoxynucleotide, which is to be placed opposite the wild-type target nucleotide, is to be either not labeled or absent. Accordingly, when the target nucleic acid is wild-type, no labeled nucleotide is added to the primer, thus giving a negative result, i.e., no signal can be detected in the primer, and the primer is not detectable. However, when the target nucleotide is mutated, a plurality of labeled nucleotides will be incorporated into the primer, and the primer is detectable. Given this distinction alone engendered by the lack of any teaching or suggestion in AG '545 of any distinction in labeling between the dideoxy- and deoxy- nucleotides, the finding that the presently claimed invention is not anticipated by the AG '545 reference is justified and warranted.

Finally, in contrast to AG '545, which requires size separation as the mutation detection method, the presently claimed invention is directed to a method of detecting or quantifying a target nucleic acid in a sample without employing gel electrophoresis size separation method, thus distinguishing the instant claims from AG '545 in this respect as well. Accordingly, for this and the other reasons discussed above, AG '545 fails to anticipate the presently claimed invention. Withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. § 103(a) Over Applied Genetics '545 in view of Shuber '778 (U.S. Patent No. 5,888,778)

Claims 16-22 have been rejected under 35 U.S.C. § 103(a) as being obvious over AG '545 in view of Shuber '778. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

AG '545 is discussed above.

Shuber '778

Shuber '778 discloses a high-throughput screening method for immobilizing nucleic acids. However, Shuber '778 fails to remedy the defects in the AG '545 publication in failing to disclose or suggest the claimed invention directed to a mutant detection method in which an unlabeled primer is hybridized immediately 3' to the target nucleotide, wherein the primer is allowed to be extended with an unlabeled dideoxynucleotide and at least one type of labeled deoxynucleotide, and further wherein the assay is not by gel electrophoresis size separation. If AG '545 and Shuber '778 were to be combined, the result would be the assay method in AG '545 with all of the inadequacies of failing to meet the limitations of claims 2-15 and 23-38, which method would now be practiced on solid-phase support. Since the addition of a solid-phase support does not remedy any of the deficiencies of AG '545 noted above, the presently claimed invention is not obvious over AG '545 and Shuber '778. Accordingly, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. § 102(e) Over Soderlund '431 (U.S. Patent No. 6,013,431)

Claims 2-38 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Soderlund '431. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Soderlund '431

Soderlund '431 is concerned with a method of determining the identity of a mutation at a target site by inserting a single labeled nucleotide opposite the target site. As a limitation on practicing the Soderlund '431 method, if the nucleotide (either the terminator or non-terminator) that complementarily matches the target nucleotide is not labeled, or if a plurality of labeled

nucleotides are incorporated into the primer at the same time, the Soderlund '431 method cannot be used to detect any variation in the target nucleotide.

Distinctions of the Claimed Invention Over Soderlund '431

Applicant submits that one of the differences between the Soderlund '431 patent and the presently claimed invention relates to the focus of each invention. In contrast to the Soderlund '431 patent disclosure, the method of the presently claimed invention is directed to a simple, rapid and accurate mutation detection method based on incorporating a plurality of nucleotides into the primer resulting in a powerful signal. In the inventive method, if the target site in the nucleic acid of interest is wild type, there is no primer extension, thus no signal. But if the target site is mutated, the primer will be extended by incorporating a plurality of labeled nucleotides.

The Examiner's attention is directed to Column 18, lines 19-46, which summarizes Soderlund '431. In particular, the Examiner's attention is directed to lines 19-20 ("A method of determining the identity of a specific nucleotide . . .") and 44-45 ("whereby the identity of the specific nucleotide at the defined site is determined"). Applicant submits that Column 18, lines 19-46 illustrates the focus of Soderlund '431.

Applicant first notes that the only way to determine the "identity of the specific nucleotide at the defined site" (emphasis added) according to Soderlund '431 is to stop the primer extension reaction after the incorporation of the one labeled nucleotide opposite the nucleotide at the defined site. This is illustrated in the Table bridging columns 17 and 18. If multiple labeled deoxynucleotides are incorporated into a primer at the same time, it would be impossible to uncover the "identity" of the target nucleotide because having a plurality of nucleotides incorporated into the primer makes it impossible to tell which nucleotide was

inserted at which position. Therefore, in Soderlund '431, only by making sure that only one base has been inserted in the primer would a person of ordinary skill in the art be confident of uncovering the identity of the target nucleotide.

Again, Applicant submits that the Soderlund '431 patent discloses and suggests adding a single labeled nucleotide opposite its target nucleotide because Soderlund '431 is concerned solely with identifying the target base. Further, Applicant notes that Soderlund '431 uses differentially labeled nucleotides. However, only one of these differentially labeled nucleotides is actually incorporated into the primer (See Table bridging columns 17 and 18). Therefore, Soderlund '431 again fails to provide any disclosure or support for incorporating a plurality of labeled nucleotides to the primer to provide a strong signal. In contrast, the present invention is simply concerned with attaining a strong signal simply to ascertain either the presence or absence of a mutation – any mutation, which is obtained by incorporating a plurality of labeled nucleotides into the primer. Depending on how many of these labeled nucleotides are incorporated or extended from the primer, the strength of the signal will be approximately proportional, twice, three times or ten times the strength of a primer incorporating only a single labeled nucleotide as expounded by Soderlund '431. Exhibit A attached herewith is presented for the Examiner's review. The photograph shows the powerful assay method of the claimed invention, which has incorporated a plurality of nucleotides (Current Invention) versus a primer incorporating only one nucleotide (Soderlund Method). The difference in the signal is noticeably powerful. For all of the reasons above, Soderlund '431 fails to anticipate the addition of a plurality of labeled nucleotides into the primer as in the present invention.

It is believed that the application is now in condition for allowance. Applicant requests the Examiner to issue a Notice of Allowance in due course. The Examiner is encouraged to contact the undersigned to further the prosecution of the present invention.

The Commissioner is authorized to charge Squire, Sanders & Dempsey's Deposit Account No. **07-1853** for any fees required under 37 CFR §§ 1.16 and 1.17 that are not covered, in whole or in part, by a check enclosed herewith and to credit any overpayment to said Deposit Account No. **07-1853**.

Respectfully submitted,

SQUIRE, SANDERS & DEMPSEY L.L.P.

Dated: August 1, 2002

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Enclosure: Exhibit A

VERSION MARKED TO SHOW CHANGES MADE

In the Claims

Please amend claim 37 as follows:

37. (Amended) A method for detecting or quantifying a target nucleic acid in a sample by detecting signal from a plurality of labeled nucleotides incorporated into a primer comprising:

(a) selecting a nucleic acid having a target nucleotide base at a predetermined position in a template of a nucleic acid of interest[, wherein the target nucleotide base in original form is not immediately adjacent on its 3' side to an identical base];

(b) preparing an unlabeled primer complementary to a sequence immediately upstream of the target nucleotide base;

(c) treating a sample containing the nucleic acid of interest, if the nucleic acid is double-stranded, so as to obtain unpaired nucleotide bases spanning the specific position, or directly employing step (d) if the nucleic acid of interest is single-stranded;

(d) annealing the primer from (b) with the target nucleic acid from (c) under high stringency conditions to obtain a primer-nucleic acid duplex, wherein the target nucleotide base in the nucleic acid of interest is the first unpaired base immediately downstream of the 3' end of the primer;

(e) mixing the primer-nucleic acid duplex from (d) with a primer extension reaction reagent comprising: (i) three types of non-terminator nucleotides that are not complementarily matched to the target nucleotide, wherein at least one type of the non-terminator nucleotide is labeled with a detectable marker; and optionally (ii) one type of

terminator nucleotide that is complementarily matched to the target nucleotide, wherein the terminator nucleotide is not labeled;

(f) performing the primer extension reaction by enzymatic or chemical means, wherein the incorporation of said non-terminator nucleotide and optionally, the terminator nucleotide, to the primer [extension] depends upon the identity of the unpaired nucleotide base in the nucleic acid template, and wherein when the target nucleotide is changed to any other type of nucleotide, a plurality of non-terminator nucleotides labeled with said detectable marker are sequence-dependently incorporated into the primer extension; and

(g) determining the presence of the [mutated nucleotide base] other type of nucleotide at the predetermined position in the nucleic acid of interest by detecting the presence of detectable signal of the non-terminator nucleotides extended from the primer, without employing gel electrophoresis size separation method.